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THE ROLE AND REGULATION OF ADENOSINE IN THE CENTRAL NERVOUS SYSTEM

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■ **Abstract** Adenosine is a modulator that has a pervasive and generally inhibitory effect on neuronal activity. Tonic activation of adenosine receptors by adenosine that is normally present in the extracellular space in brain tissue leads to inhibitory effects that appear to be mediated by both adenosine A_1 and A_{2A} receptors. Relief from this tonic inhibition by receptor antagonists such as caffeine accounts for the excitatory actions of these agents. Characterization of the effects of adenosine receptor agonists and antagonists has led to numerous hypotheses concerning the role of this nucleoside. Previous work has established a role for adenosine in a diverse array of neural phenomena, which include regulation of sleep and the level of arousal, neuroprotection, regulation of seizure susceptibility, locomotor effects, analgesia, mediation of the effects of ethanol, and chronic drug use.

INTRODUCTION

Purines and purine nucleotides are essential constituents of all living cells. ATP is used as an energy source for nearly all cellular activity, whereas adenine is a component of nucleic acids. Perhaps as a result of their ubiquitous nature, purines have also evolved as important molecules for both intracellular and extracellular signaling, roles that are distinct from their activity related to energy metabolism and the genetic transmission of information. ATP itself interacts with two general classes of extracellular receptors, the ionotropic P2X receptors and the metabotropic P2Y receptors (for reviews, see Ralevic & Burnstock 1998, Harden et al 1995), and cAMP is an intracellular messenger that plays a key role in regulating intracellular activity. Adenosine is a third "purinergic messenger" that regulates many physiological processes, particularly in excitable tissues such as heart and brain. Many of the actions of adenosine either reduce the activity of excitable tissues (e.g. by

slowing the heart rate) or increase the delivery of metabolic substrates (e.g. by inducing vasodilation) and, thus, help to couple the rate of energy expenditure to the energy supply. However, this type of unitary role for adenosine is not sufficient to explain many of its actions, and it is clear that adenosine plays a variety of different roles as an intercellular messenger. This is particularly the case in the brain, which expresses high concentrations of adenosine receptors, and where adenosine has been shown to be involved in both normal and pathophysiological processes, including regulation of sleep, arousal, neuroprotection, and epilepsy. The pharmacological actions of caffeine, which is the most widely used psychoactive drug in the world, are largely attributable to its activity as an adenosine receptor antagonist (Fredholm et al 1999). A challenging issue with respect to the functional role(s) played by adenosine in the brain is to understand why antagonizing the effects of endogenous adenosine produce what are generally considered to be improvements in mental function and performance, whereas antagonism of most other neurotransmitter receptors produce either deficits or pathological effects. The primary intent of this review is to explore the functional role of adenosine in the nervous system and to discuss the mechanisms by which extracellular concentrations of adenosine are regulated.

ADENOSINE RECEPTORS AND TRANSDUCTION MECHANISMS

Adenosine Receptor Subtypes

Adenosine receptors have been intensively studied, and to date four different adenosine receptors have been cloned in a variety of species, including man (Table 1) (for a review, see Olah & Stiles 1995). Because exhaustive efforts to identify other adenosine receptors have been unsuccessful, it appears unlikely that additional receptors will be identified. All of the adenosine receptors are seven transmembrane domain, G-protein–coupled receptors, and they are linked to a variety of transduction mechanisms. The A_1 receptor has the highest abundance in the brain and is coupled to activation of K^+ channels (Trussell & Jackson 1985) and inhibition of Ca^{2+} channels (Macdonald et al 1986), both of which would inhibit neuronal activity. The A_{2A} receptor is expressed at high levels in only a few regions of the brain and is primarily linked to activation of adenylyl cyclase. Antagonism of both A_1 and A_{2A} receptors appears to be responsible for the stimulant effects of adenosine receptor antagonists, at least in rodents (Marston et al 1998), although stimulation of locomotor activity may be primarily an A_{2A} effect (Ongini 1997, El Yacoubi et al 2000). The A_{2B} receptor, which also activates adenylyl cyclase, is thought to be fairly ubiquitous in the brain, but it has been difficult to link this receptor to specific physiological or behavioral responses because of the paucity of A_{2B}-specific agonists or antagonists (for a review, see Feoktistov & Biaggioni 1997). The A_3 receptor is also somewhat poorly characterized, but it has been

TABLE 1 Adenosine receptors in the brain

Receptor	Adenosine affinity	G-protein	Transduction mechanisms[a]	Physiological actions in brain	Distribution in brain
A_1	~70 nM	G_i and G_o	Inhibits adenylyl cyclase Activates GIRKs Inhibits Ca^{2+} channels Activates PLC	Inhibits synaptic transmission Hyperpolarizes neurons	Widespread[b]
A_{2A}	~150 nM	G_s[c], G_{olf}	Activates adenylyl cyclase Inhibits Ca^{2+} channels Activates Ca^{2+} channels (?)	Facilitates transmitter release[d] Inhibition of transmitter release[d]	Primarily striatum, olfactory tubercle, nucleus accumbens[e]
A_{2B}	~5100 nM	G_s[c]	Activates adenylyl cyclase Activates PLC	Increases in cAMP in brain slices Modulation of Ca^{2+} channel function (?)	Widespread[f]
A_3	~6500 nM	G_{i3}, G_q	Activates PLC Inhibits adenylyl cyclase Increases intracellular Ca^{2+}	Uncouples A_1, mGlu receptors (?)	Widespread[f]

[a]GIRKs, G-protein–dependent inwardly rectifying K^+ channels; PLC, phospholipase C.

[b]Based upon numerous ligand binding studies and in situ hybridization studies.

[c]Primary mechanism of coupling.

[d]For reveiws, see Latini et al 1996, Edwards & Robertson 1999.

[e]Ligand binding and in situ hybridization generally shows high levels in these regions, very low levels elsewhere; reverse transcriptase–polymerase chain reaction (RT-PCR) shows more widespread expression.

[f]Relatively low levels, not detectable with in situ hybridization but apparent with RT-PCR (Dixon et al 1996).

reported to uncouple A_1 and metabotropic glutamate receptors via a protein kinase C–dependent mechanism (Dunwiddie et al 1997a, Macek et al 1998), and thus, one of its functions may be to modulate the activity of other receptors.

From a pharmacological standpoint, it has been extremely difficult to develop tissue-specific drugs that interact with adenosine receptors, primarily because of their ubiquitous nature. For example, although there are highly A_1-selective agonists and antagonists, the A_1 receptor that slows the heart rate appears to be identical to the A_1 receptor that depresses neural activity. Although there may be tissue differences in spare receptors (Shryock et al 1998), G-protein coupling, and transduction mechanisms (Linden et al 1998), there are few differences that can be exploited pharmacologically.

ACTIONS OF ADENOSINE AT THE CELLULAR LEVEL

Actions of Adenosine Mediated by Effects on K^+ and Ca^{2+} Channels

In terms of cellular physiology, adenosine has a number of actions that would be considered neuromodulatory but not neurotransmission per se. Adenosine does not appear to be released in a classical Ca^{2+}-dependent fashion, nor is it stored in vesicles, and there is no evidence for synapses where the primary transmitter is adenosine. However, A_1 receptors are linked to inhibition of the release of virtually every classical neurotransmitter (including glutamate, gamma-aminobutyric acid (GABA), acetylcholine, norepinephrine, 5-hydroxytryptamine (5-HT), dopamine, and other transmitters as well). The most prominent inhibitory actions are generally on excitatory glutamatergic systems (e.g. Dunwiddie & Hoffer 1980, Kocsis et al 1984), where synaptic transmission can often be completely blocked by adenosine. Inhibitory modulation of inhibitory (e.g. GABA) systems is less frequently observed, so that the net effect of adenosine receptor activation in nearly all regions of the brain is to reduce excitability. The mechanism of inhibitory modulation of transmitter release has been extensively studied, and it appears to reflect a G-protein–coupled inhibition of Ca^{2+} channels in nerve endings, although this is still the subject of debate. Other mechanisms may contribute to this effect as well, because adenosine also inhibits the spontaneous Ca^{2+}-independent release of neurotransmitter (Scanziani et al 1992), but under normal physiological conditions the inhibition of Ca^{2+} influx appears to be the primary inhibitory mechanism (Fredholm & Dunwiddie 1988, Wu & Saggau 1997). Adenosine receptors may also enhance neurotransmitter release (Cunha et al 1994), but these actions are less common than the inhibition of neurotransmitter release. Another major action of A_1 receptors is a hyperpolarization of the resting membrane potential mediated via a G-protein–dependent activation of inwardly rectifying K^+ channels (GIRKs). GIRKs are activated by many other receptors as well (e.g. in hippocampal pyramidal neurons by A_1, $GABA_B$, $5HT_{1A}$, and somatostatin receptors), and

the effects of these agents typically occlude, which suggests that they act on a common population of G-proteins and/or K^+ channels.

Interactions Between Adenosine Receptors and Other Receptor Systems

One interesting aspect of adenosine receptors pertains to interactions between adenosine receptors and other types of G-protein–coupled receptors. Synergistic interactions have been reported between low concentrations of A_1 and $GABA_B$ agonists on GIRKs (Sodickson & Bean 1998), which suggests that the tonic, low level of occupation of A_1 receptors might regulate the strength of $GABA_B$ synapses. There is also extensive evidence from studies primarily in the striatum for direct interactions between A_{2A} receptors and D1 receptors, and between A_1 and D2 receptors (for a review, see Fuxe et al 1998).

REGULATION OF EXTRACELLULAR ADENOSINE

In many systems, basal extracellular adenosine concentrations are sufficient to tonically activate a substantial fraction of high-affinity (A_1 and A_{2A}) adenosine receptors. Estimates of this basal concentration span a wide range, but most estimates using pharmacological approaches (Dunwiddie & Diao 1994) or microdialysis of the brain (Ballarin et al 1991) are in the range of 25–250 nM. Given the affinity of adenosine for its receptors (Table 1), this would suggest that interactions with A_1 and A_{2A} receptors are primarily responsible for the basal purinergic "tone" that is seen in most systems. The stimulatory effects of such drugs as caffeine stem from their ability to antagonize the actions of endogenous adenosine and, hence, reverse this tonic inhibition. Little is known about this basal tone, which in the brain may differ markedly from region to region (Delaney & Geiger 1996). Basal concentrations of adenosine probably reflect an equilibrium between the multiple mechanisms that increase extracellular adenosine and its uptake and metabolism. The recent observation that A_1 receptors appear to play a role in the regulation of adenosine concentrations in neuronal cultures (Andresen et al 1999) suggests that there may be some interesting unknown aspects to the regulation of extracellular adenosine concentrations.

Extracellular Conversion of Adenine Nucleotides as a Source for Adenosine

There are two primary mechanisms by which adenosine can reach the extracellular space of the brain, and these are via dephosphorylation of adenine nucleotides by ecto-nucleotidases and release of adenosine from cells via transporters. The first of these depends on ecto-nucleotidases, ecto-phosphodiesterases, and apyrases that can dephosphorylate virtually any adenine nucleotide to 5′-AMP, which is

subsequently dephosphorylated by 5′-nucleotidase to adenosine. There are a wide variety of such ectonucleotidases, which have been the subject of a recent review (Zimmermann & Braun 1999). These ecto-enzymes are highly expressed in the brain, have rather broad specificity, and are generally rapid in their action. Recent studies have suggested that most nucleotides (with the exception of cAMP) are converted to adenosine in less than a second (Dunwiddie et al 1997b). Even "stable" ATP analogs can be substrates for these nucleotidases (Cunha et al 1998), and there is evidence that the nucleotidases may be present in close physical proximity to presynaptic inhibitory A_1 receptors.

There are multiple mechanisms by which adenine nucleotides are known to be released into the extracellular space. ATP is colocalized with such neurotransmitters as acetylcholine, dopamine, 5-HT, and norepinephrine, and is coreleased on electrical stimulation (e.g. White 1977, Fredholm et al 1982), where it is subsequently hydrolyzed to adenosine. In many systems, cAMP is released into the extracellular space by a probenecid-sensitive transporter (Rosenberg & Li 1995). The amounts of cAMP released in this fashion are sufficient to produce large increases in extracellular adenosine. This can be observed with forskolin stimulation of adenylyl cyclase (Dunwiddie et al 1992, Brundege et al 1997) and following receptor-mediated activation of adenylyl cyclase (Gereau & Conn 1994).

There may be yet other mechanisms for nucleotide release in the brain as well. Proteins that are members of the ATP-binding cassette family of proteins, such as P-glycoprotein (Abraham et al 1993) and the cystic fibrosis transmembrane conductance regulator (Prat et al 1996), appear to be able to function as ATP-conducting ion channels, although this has not been demonstrated in the brain. ATP can also be released by activation of stretch-activated receptors (Hazama et al 1999).

Release of Adenosine Via Facilitated Diffusion Transporters

Another mechanism by which adenosine levels in the extracellular space are regulated is by facilitated diffusion nucleoside transporters. There are two known forms of this transporter, which have been distinguished by their sensitivity to the transport inhibitor nitrobenzylthioinosine (for a review, see Cass et al 1998). These transporters are passive, in that they do not depend on ATP or ionic gradients to transport adenosine, and they equilibrate the concentration of adenosine across cellular membranes. Because of the relatively high activity of intracellular adenosine kinase, adenosine concentrations inside cells are normally low, so the net flux through these transporters is inwardly directed. However, under conditions where intracellular adenosine concentrations rise, these transporters can release adenosine. There are also active transport mechanisms for adenosine, which depend on the Na^+ gradient to provide the energy for transport. Some of these transporters have been cloned (Cass et al 1998), but their relative importance in the regulation of extracellular adenosine concentrations is unclear, largely because of a lack of selective pharmacological tools for these transporters. It is also possible that these

transporters could be driven in reverse when intracellular adenosine is high and the Na^+ gradient is reduced, such as during hypoxia, ischemia, and seizures, and thus could become mechanisms for adenosine release as well.

Regulation of Intracellular Adenosine

Because of the presence of equilibrative transporters, regulation of intracellular adenosine concentrations is critical to the regulation of extracellular adenosine, and this control is exerted in two ways. First, if intracellular concentrations of adenosine rise, the ability of these transporters to take up adenosine formed extracellularly from nucleotides is lost, as the adenosine gradient is reduced. Second, if intracellular adenosine concentrations rise even further, direct efflux of adenosine will occur when the intracellular concentration of adenosine exceeds the extracellular.

Although the basic metabolic pathways for intracellular nucleotides in the brain are known, the precise regulation of adenosine kinase and cytosolic 5′-nucleotidase is not well understood (Figure 1). In other tissues, such as heart (Kroll et al 1993), and in hepatocytes (Bontemps et al 1983), there is a high rate of flux in a futile cycle involving these two enzymes, and partly as a consequence, inhibition of adenosine kinase in the heart (and in the brain) leads to very large increases in adenosine.

Physiological Stimuli that Release Adenosine in the Brain

Many physiological manipulations can increase extracellular adenosine, often by cellular mechanisms that are not well understood (Table 2). It would appear likely that the regulation of the activity of key enzymes in intracellular adenine nucleotide/adenosine metabolism (cytosolic 5′ nucleotidase-I and adenosine kinase, but also possibly S-adenosylhomocysteine hydrolase and adenosine deaminase) is central to the mechanisms by which diverse stimuli elevate extracellular adenosine in the brain. The diversion of adenine nucleotides into the AMP-adenosine cycle (e.g. by the breakdown of ATP to ADP and AMP during ischemia) would also be expected to contribute to increases in adenosine by mass action, independently of any regulation of enzyme activity.

Although there is wide diversity in the stimuli that will release adenosine, there seem to be some common elements. Manipulations that cause the energy requirements of brain to outstrip its ability to synthesize ATP profoundly increase adenosine release. This can occur either through a large increase in energy requirements (e.g. during seizures) or because of a loss of metabolic substrates (e.g. ischemia). Under these conditions, ATP levels are reduced, and the levels of other adenine nucleotides and adenosine are increased. Because intracellular ATP concentrations are high (typically estimated to be in the range of 3 mM), even a 1% conversion of ATP to adenosine would result in an approximate 100-fold increase in intracellular adenosine and a corresponding increase in the extracellular concentration. However, there is also evidence that adenosine can be released

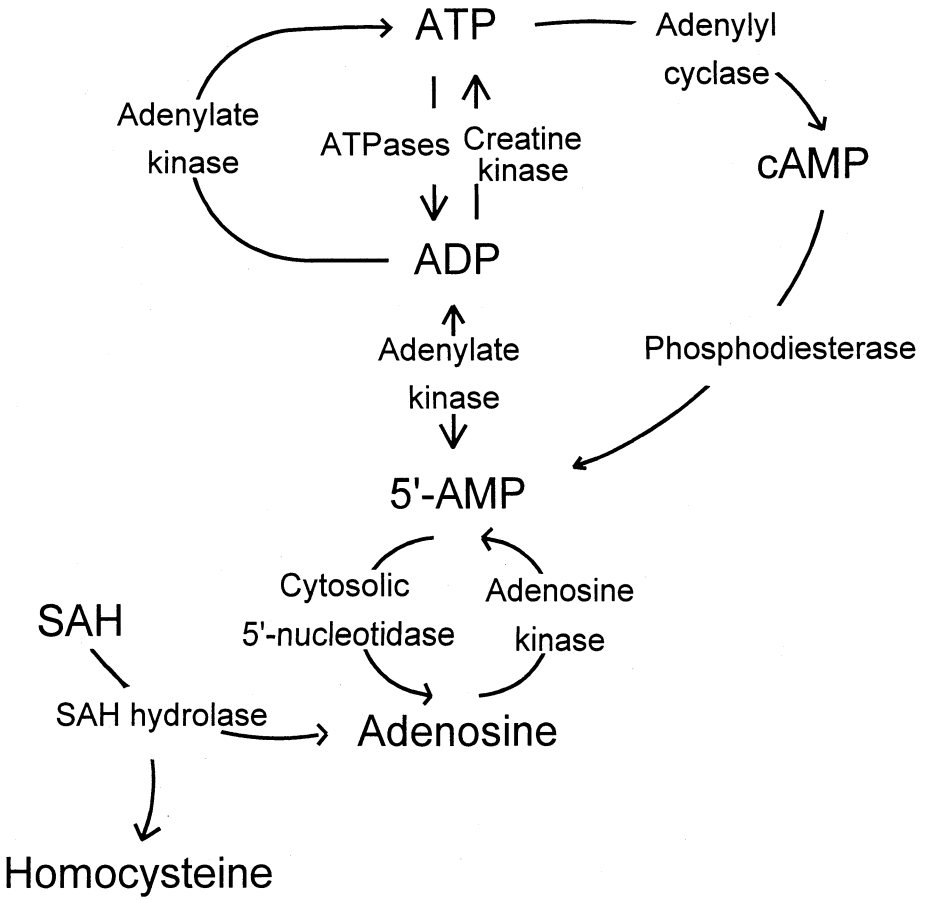

Figure 1 The primary intracellular pathways for the formation of adenosine. Adenosine is formed from 5′-AMP by the cytosolic 5′-nucleotidase and is converted back to 5′-AMP by adenosine kinase (which requires ATP as a phosphate donor). Under resting conditions, there is often a substantial flux through this futile cycle. Adenosine may also be formed by the action of S-adenosylhomocysteine (SAH) hydrolase.

under conditions that should preserve ATP levels (Doolette 1997). Similarly, inhibition of adenosine kinase probably has little effect on ATP levels, but it profoundly increases adenosine release (Pak et al 1994, Lloyd & Fredholm 1995, Brundege & Dunwiddie 1998). In the heart, hypoxia produces a profound inhibition of adenosine kinase activity (to as low as 6% of normal activity) (Decking et al 1997), whereas 5′-nucleotidase activity does not appear to be greatly affected. This generates large amounts of adenosine, and a similar mechanism might underlie adenosine release in the brain as well.

TABLE 2 Experimental manipulations that stimulate adenosine release in the brain[a]

Stimulus[b]	References
Physiological	
Hypoxia, anoxia	Fowler 1989,1993a,b; Gribkoff et al 1990; Lloyd et al 1993; Zetterström et al 1982; Zhu & Krnjevic 1994
Ischemia	Fowler 1993a,b; Pedata et al 1993; Phillis et al 1987
Hypoglycemia	Fowler 1993a,b
Seizures	During & Spencer 1993, Lewin & Bleck 1981, Schrader et al 1980, Winn et al 1980
Increases in temperature	Gabriel et al 1998, Masino & Dunwiddie 1999
Free radicals	Delaney et al 1998, Masino et al 1999
Electrical stimulation	Lloyd et al 1993, Pull & McIlwain 1972, Schrader et al 1980, Yawo & Chuhma 1993
K^+ depolarization	Hoehn & White 1990
Synaptic stimulation	Grover & Teyler 1993, Manzoni et al 1994, Mitchell et al 1993
Pharmacological	
Adenosine kinase inhibitors	Brundege & Dunwiddie 1998, Doolette 1997, Lloyd & Fredholm 1995, Pak et al 1994
Lipopolysaccharides, interleukin-1β	Luk et al 1999, Wang & White 1999
Intracellular acidification	SA Masino, unpublished data
Metabolic inhibitors	
Cyanide	Doolette 1997
Dinitrophenol	Doolette 1997
Na^+ replacement	Fowler 1995
Opiate receptor activation	Stone et al 1989, Sweeney et al 1991
AMPA receptor activation	
Increase	Craig & White 1993
No change	Delaney et al 1998
Kainate receptor activation	Craig & White 1993, Delaney et al 1998
NMDA receptor activation	Chen et al 1992, Craig & White 1993, Delaney et al 1998, Manzoni et al 1994
5-HT receptor activation	Sweeney et al 1990
Forskolin (via cAMP)	Brundege & Dunwiddie 1998, Dunwiddie et al 1992

[a]The preceding list includes not only agents that are thought to directly lead to the efflux of adenosine, but also ones that may release an adenine nucleotide that is subsequently converted to adenosine [e.g. N-methyl-D-aspartate (NMDA) receptor activation). In addition, some of these stimuli may act indirectly via other mechanisms (e.g. K^+ stimulated release is at least partially the result of glutamate release) (Hoehn & White 1990).

[b]AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; 5-HT, 5-hydroxytryptamine.

There are yet other kinds of stimuli [e.g. N-methyl-D-aspartate (NMDA) receptor activation], where the cellular mechanism underlying the release is largely unknown, and where increased ATP breakdown seems unlikely to account for release. Biochemical experiments have suggested that NMDA receptor activation releases an unknown nucleotide, which is then converted to adenosine (Craig & White 1993), whereas electrophysiological experiments have suggested that activation of NMDA receptors releases adenosine per se (Manzoni et al 1994). It seems unlikely that a common mechanism could account for release under all of these conditions, although Doolette (1997) has suggested that intracellular acidification, which can be induced by nearly all the stimuli listed in Table 2, might be a common factor. Further experiments will be required to evaluate the merit of this hypothesis.

Removal of Adenosine from the Extracellular Space

The mechanisms that are responsible for clearing adenosine from the extracellular space are not completely understood, but the transport of adenosine into cells, either by facilitated diffusion or by active transport, appears to be the primary mechanism. Inhibition of facilitated diffusion transport leads to a slowly developing but substantial increase in extracellular adenosine (Dunwiddie & Diao 1994, Zhu & Krnjevic 1994). These increases would probably occur more rapidly except for the fact that inhibitors of transport inhibit both efflux as well as uptake. Thus, the adenosine that builds up extracellularly must come from other sources, such as metabolism of nucleotides by ecto-nucleotidases.

An alternative pathway for the inactivation of extracellular adenosine is its metabolic transformation to inosine by adenosine deaminase. Under either basal conditions or during stimulated release of adenosine from slices of brain tissue, adenosine usually comprises $<10\%$ of the total purine efflux, whereas the remainder appears as the adenosine metabolites inosine, hypoxanthine, or xanthine (Pedata et al 1990, Lloyd et al 1993). Although this might imply that adenosine deaminase is relatively important in clearing the extracellular space of adenosine, this is not the case. Adenosine deaminase inhibitors have little or no influence on the concentration of extracellular adenosine (Pak et al 1994, Zhu & Krnjevic 1994; TV Dunwiddie, unpublished data), whereas uptake inhibitors substantially increase adenosine concentrations (Dunwiddie & Diao 1994). The resolution of these seemingly paradoxical observations is that the majority of adenosine in the extracellular space is cleared via reuptake; however, any metabolites that are formed are much more likely than adenosine to diffuse out of the slice without being recaptured and, hence, make a disproportionate contribution to purine efflux. Nevertheless, during hypoxia and ischemia, adenosine deaminase assumes a prominent role in regulating extracellular adenosine concentrations (Lloyd & Fredholm 1995, Barankiewicz et al 1997, Dupere et al 1999). Under these conditions, the adenosine transporters probably are largely inactive, so adenosine deaminase becomes important in the absence of any other mechanisms for adenosine removal.

Abnormalities in Adenosine Regulation

Little is known about the possibility that levels of extracellular adenosine in the brain may differ between individuals, and possibly in certain disease states. However, it has been known for some time that in Down syndrome, purine levels are generally elevated by approximately 50% (Pant et al 1968). Most notable, AMP and ADP levels are significantly elevated, whereas ATP is not (Stocchi et al 1985). A number of the known abnormalities in Down syndrome, such as daytime sedation/sleepiness, reduced pain sensitivity (Martinez-Cue et al 1999), learning disorders (Siarey et al 1999), and central sleep apneas (Ferri et al 1997), are consistent with increased adenosine concentrations, because in experimental models adenosine can produce all these effects. One prediction based on these observations would be that there might be an elevated sensitivity to adenosine receptor antagonists in Down syndrome, but this has apparently never been tested.

PHYSIOLOGICAL ROLES OF ADENOSINE

Role of Adenosine in Normal Physiology

Adenosine appears to subserve a number of diverse roles in normal physiology, which include promoting and/or maintaining sleep, regulating the general state of arousal as well as local neuronal excitability, and coupling cerebral blood flow to energy demand. Selective adenosine receptor antagonists have been used frequently in the past to provide evidence concerning these proposed roles for adenosine. The more recent development of knockout mice for the A_{2A} receptor (Ledent et al 1997, Chen et al 1999), A_3 receptor (Zhao et al 2000), and A_1 receptor (BB Fredholm, personal communication) have provided additional tools with which to characterize the functions of these receptors.

Sleep and Regulation of Arousal The idea that adenosine plays a role in sleep is a natural outgrowth of the observation that adenosine receptor antagonists such as caffeine promote wakefulness and disrupt normal sleep. Evidence to support this hypothesis generally has fallen into two categories. First, direct measurement of endogenous adenosine in the basal forebrain of cats using microdialysis has shown that adenosine levels progressively increase during prolonged wakefulness and decrease during subsequent recovery sleep (Porkka-Heiskanen et al 1997, Porkka-Heiskanen 1999). A similar relationship between behavioral state and endogenous adenosine appears to exist in the hippocampus but not in the thalamus (Huston et al 1996). Second, pharmacological manipulations involving adenosine receptors have shown that agonists generally promote sleep (Portas et al 1997), whereas antagonists reduce sleep (Lin et al 1997). Some of the most compelling evidence along these lines comes from studies showing that adenosine inhibits neuronal activity in cholinergic nuclei that are thought to regulate arousal (Rainnie et al 1994), and that adenosine dialysis into these regions in vivo promotes sleep and reduces the level

of arousal as measured by EEG activity (Portas et al 1997). Parallel noncholinergic systems that contribute to sleep regulation may exist as well; for example, infusion of adenosine or a selective A_1 agonist into the preoptic area has been shown to reduce sleep latency, increase total sleep time, and increase slow-wave sleep (Ticho & Radulovacki 1991, Mendelson 2000). Although many studies have implicated A_1 receptors in both sleep and decreased arousal (Dunwiddie & Worth 1982, Fulga & Stone 1998), there is also evidence that A_{2A} receptors may be involved, particularly in the rostral basal forebrain, where the A_{2A} agonist CGS21680 promotes both REM (rapid eye movement) and non-REM sleep (Satoh et al 1999).

Adenosine as a Retrograde Synaptic Messenger Although adenosine does not appear to be a classical neurotransmitter, there is some evidence that adenosine could serve as a retrograde synaptic messenger. If an individual neuron is loaded with adenosine via patch pipette, adenosine efflux from that cell is sufficient to significantly inhibit its synaptic inputs, whereas synaptic communication to other nearby cells is unaffected (Brundege & Dunwiddie 1996). The precise subcellular localization of these transporters would be important with respect to this kind of speculative mechanism. However, previous localization studies regarding these transporters are not definitive, or they have lacked the resolution necessary to evaluate this possibility. However, now that these transporters have been cloned, the distribution of transporters should be characterized with more precision and resolution.

Adenosine as a Mechanism for Coupling Energy Demand to Cerebral Blood Flow Adenosine has long been recognized to be involved in the autoregulation of cerebral blood flow (Berne et al 1974, Winn et al 1981, Wahl & Schilling 1993), where it modulates vascular resistance via A_{2A} receptors (Phillis 1989, Coney & Marshall 1998). Adenosine applied externally to cerebral blood vessels induces vasodilation (Hylland et al 1994), and there is evidence that endogenous adenosine is a tonic regulator of vascular smooth muscle tone. Thus, application of adenosine antagonists causes vasoconstriction and reverses adenosine-mediated vasodilation (Ko et al 1990, Dirnagl et al 1994, Hylland et al 1994). Accordingly, any stimulus that promotes release of additional adenosine from neurons or glia will induce vasodilation.

It has been suggested that this relationship between adenosine and cerebral blood flow is a mechanism that couples increased cell energy expenditure (seen as increased ATP utilization and demand) with increased oxygen and glucose delivery via the cerebral vasculature. The increased adenosine released during such conditions as ischemia would serve to increase cerebral blood flow and could ameliorate the effects of ischemia. However, nonpathological changes in energy requirements have a similar effect; for example, increased activity in somatosensory cortex due to peripheral sensory stimulation is sufficient to induce vasodilation mediated via adenosine, indicating that adenosine is a component of the autoregulatory mechanisms that act on the cerebral vasculature (Ko et al 1990, Dirnagl et al 1994).

Role of Adenosine in Pathological Conditions

Extracellular brain concentrations of adenosine are markedly elevated by a diverse array of pathological stimuli (Table 2). Many of the effects of adenosine that are observed to a minor extent under normal conditions (e.g. presynaptic inhibition of glutamate release) are greatly augmented during pathological events and are neuroprotective in that context. In addition to having acute protective effects, transient activation of adenosine receptors offers protection against damage induced by a subsequent hypoxic or ischemic event. This phenomenon, which is referred to as preconditioning, occurs not only in brain but also in other excitable tissues, such as heart (Miura & Tsuchida 1999).

Neuroprotective Effects of Adenosine in Hypoxia and Ischemia *Acute protective effects* Endogenous adenosine released by hypoxia (Gribkoff & Bauman 1992, Fowler 1993a,b), ischemia (Lloyd et al 1993, Latini et al 1999), electrical activity (Arvin et al 1989, Lloyd et al 1993), and hypo- or aglycemia (Fowler 1993b, Hsu et al 1994, Calabresi et al 1997) reduces the subsequent damage to neuronal tissue. This neuroprotection offered by adenosine is also effective against other kinds of damage that are not as directly related to energy metabolism, such as mechanical cell injury (Mitchell et al 1995) and methamphetamine-induced neurotoxicity (Delle Donne & Sonsalla 1994). Conversely, applying adenosine receptor antagonists in conjunction with any of these conditions exacerbates the consequent damage (Arvin et al 1989, Hsu et al 1994, Mitchell et al 1995).

The neuroprotective actions of adenosine are mediated primarily via A_1 receptor activation, and at least three cellular mechanisms may be involved. Adenosine strongly inhibits transmitter release (and glutamate in particular), hyperpolarizes neurons, and directly inhibits certain kinds of Ca^{2+} channels. All these actions could reduce excitotoxicity by limiting Ca^{2+} entry, which is thought to be a key step in excitotoxic damage, and by reducing metabolic demand, which would help to preserve ATP stores that are essential for pumping Ca^{2+} out of the cell. Experiments with cardiac tissue suggest that the number of A_1 receptors may be a limiting factor in acute protection because overexpression of A_1 receptors provides additional protection against ischemia-reperfusion injury (Matherne et al 1997, Headrick et al 1998). A similar protective effect may be possible in neuronal tissue because an allosteric enhancer of A_1 receptor binding has been shown to offer neuroprotection in neonates (Halle et al 1997). The utility of an alternative strategy, i.e. enhancing the local release of adenosine (e.g. by inhibiting adenosine kinase), is not clear, although positive effects have been reported (Jiang et al 1997). The concentrations of adenosine in the extracellular space during ischemia probably saturate A_1 receptors, so the primary effect of enhancing adenosine release would be expected to be in marginally affected regions, where adenosine concentrations are not as high.

Alternatively, some of the protective effects could be mediated by other receptors (e.g. the A_3 receptor), which has a substantially lower affinity for adenosine

and thus, would require higher concentrations for maximal activation. The A_{2A} receptor, on the other hand, may actually contribute to ischemic tissue damage, because mice lacking A_{2A} receptors show reduced brain damage following focal ischemia (Chen et al 1999). The neuroprotective role of adenosine has been reviewed recently (Deckert & Gleiter 1994, Schubert et al 1997, Fredholm 1997) and continues to be an area of rapid development.

Preconditioning A brief episode of mild hypoxia or ischemia that produces little or no damage has been shown to afford protection against a subsequent challenge of greater severity presented hours or even days later. This effect, which is observed in both cardiac and neuronal tissues, has been termed preconditioning and seems to involve A_3 as well as A_1 receptors (Stambaugh et al 1997, Liang & Jacobson 1998). In the brain, adenosine release, A_1 receptor activation, and the opening of ATP-dependent K^+ channels appear to play a central role in preconditioning (Heurteaux et al 1995). Recently, it has been observed that cross-tolerance exists between potentially damaging stimuli, and many of these interactions involve adenosine receptors. For example, a sublethal kainate seizure will protect against subsequent ischemia, and vice versa (Plamondon et al 1999). Chemical inhibition of oxidative phosphorylation provides protection against hypoxia within an hour and lasts for 24 h (Riepe et al 1997), and it may protect against other insults as well. Much clinical interest is focused on determining how to maximize acute neuroprotection, and how to take advantage of the preconditioning phenomenon in both the brain and the heart to improve patient outcome (Liang & Jacobson 1999, Schwarz et al 1999).

Epilepsy Consistent with its role as an inhibitory neuromodulator, adenosine exhibits anticonvulsant effects in experimental models of epilepsy (for a recent review, see Dunwiddie 1999b). Exogenously administered adenosine receptor agonists reduce seizure activity (Dunwiddie & Worth 1982, Barraco et al 1984, Zhang et al 1990), whereas adenosine receptor antagonists have proconvulsant effects (Dunwiddie 1980, Ault et al 1987), which in hippocampus are mediated by A_1 receptors (Alzheimer et al 1989). Because endogenous levels of adenosine rise markedly during seizure activity (Table 2), it has been proposed that adenosine functions as an "endogenous anticonvulsant" (Dragunow 1988). However, neither the loss of A_1 receptors in knockout mice (BB Fredholm, personal communication) nor the antagonism of adenosine receptors by such antagonists as caffeine lead directly to seizures. Very high concentrations of caffeine can induce convulsions, but this occurs in concentrations where actions other than adenosine receptor antagonism are probably involved. The anticonvulsant effects of adenosine appear to be mediated primarily by A_1 receptors (Murray et al 1992, Zhang et al 1994), although there may be A_{2A} involvement in some regions of the brain. Audiogenic seizures in DBA/2 mice are inhibited by both A_1 and A_{2A} receptor agonists, and selective antagonists for each subtype promote seizures (De Sarro et al 1999).

Beyond the acute anticonvulsant effects of adenosine acting at A_1 receptors, a chronic reduction of A_1 receptors has been found in epileptic tissue, in both humans (Glass et al 1996) and rats (Ochiishi et al 1999). A loss of the tonic inhibitory effects

of adenosine may contribute to the hyperexcitability and recurrent seizures that characterize epilepsy.

Despite its profound anticonvulsant effects, adenosine agonists have not proved clinically useful in the treatment of epilepsy because of the peripheral effects of adenosine, which include decreased heart rate, blood pressure, and body temperature (Dunwiddie 1999b). Effective strategies that enhance the protective effects of adenosine near a seizure focus may require a novel approach, such as a method for local release of adenosine. Using this type of technique, Boison et al (1999) have produced a profound reduction in seizure activity in kindled animals by implanting an adenosine-releasing polymer into the cerebral ventricle. Alternatively, a pharmacological strategy that potentiates the effect of endogenous adenosine, such as inhibiting adenosine kinase (Kowaluk & Jarvis 2000) may have clinical potential.

Adenosine and the Actions of Drugs of Abuse

As discussed above, adenosine is centrally involved in the actions of caffeine, which is a relatively nonselective adenosine receptor antagonist, and this pharmacological action is largely responsible for the effects of caffeine on the central nervous system (Fredholm et al 1999). However, there is also evidence that the effects of drugs of abuse may be linked in some manner to adenosine as well.

Ethanol Among the various drugs of abuse, ethanol is perhaps most closely linked mechanistically to adenosine. Three general mechanisms have been proposed to account for this interaction, involving changes in adenosine formation, adenosine uptake, and effects on adenosine receptor coupling. One potential interaction relates to the fact that substantial concentrations (1–2 mM) of acetate are formed as a result of the metabolism of ethanol (Carmichael et al 1991), which is then incorporated into acetyl-coenzyme A with the concomitant formation of AMP. The increase in AMP could then lead directly to increased adenosine formation (Figure 1). Ethanol has also been reported to inhibit facilitated diffusion transporters (Diamond et al 1991, Krauss et al 1993), which would increase extracellular brain concentrations of adenosine by inhibiting uptake. Finally, ethanol can facilitate the receptor-mediated activation of adenylyl cyclase by various hormones and neurotransmitters (Rabin & Molinoff 1981, Hoffman & Tabakoff 1990). Because all the known adenosine receptors can interact with adenylyl cyclase, this provides a third mechanism by which ethanol could modulate effects mediated via adenosine receptors. The general subject of ethanol-adenosine interactions has been discussed extensively in a recent review (Dunwiddie 1999a).

Opiates A number of studies have suggested that opioids in particular, and possibly psychomotor stimulants such as cocaine as well, can interact with adenosine systems. As far as the opioids are concerned, agonists such as morphine have been shown to release adenosine in the brain, spinal cord, and peripheral nervous system (Fredholm & Vernet 1978, Stone 1981, Cahill et al 1996), and this release

occurs via a facilitated diffusion transporter (Sweeney et al 1993). The early observation that opioid analgesia can be at least partially antagonized by adenosine receptor antagonists (Ho et al 1973) has been confirmed in more recent studies as well (for a review, see Sawynok et al 1989) and has led to the hypothesis that some opiate actions are mediated indirectly via release of adenosine. The well-established analgesic properties of adenosine receptor agonists (Herrick-Davis et al 1989, Sosnowski et al 1989, Sawynok 1998) provide further support for this hypothesis. In behavioral studies, adenosine receptor antagonists elicit a response termed the quasi-morphine withdrawal syndrome (Francis et al 1975), which in many respects is similar to the response to naloxone in opiate-tolerant animals. Thus, there are strong parallels between the pharmacological effects of opiate and adenosine agonists, and also between opiate and adenosine antagonists. Finally, an interesting purinergic role has also emerged in terms of the effects of chronic opioids as well as cocaine; in animals withdrawn from chronic treatment with either morphine or cocaine, there are persistent increases in extracellular adenosine in the ventral tegmental region, a brain region intimately involved in the rewarding effects of these drugs (Bonci & Williams 1996, Shoji et al 1999, Fiorillo & Williams 2000). The source of this adenosine appears to be from the release of cAMP and subsequent extracellular catabolism to adenosine.

SUMMARY AND CONCLUSIONS

Adenosine is involved in a diverse array of functions in the central nervous system. Although in a general sense many of its effects are inhibitory, consistent with its proposed roles as an endogenous anticonvulsant, neuroprotectant, and sleep-inducing factor, this differs depending on the brain system and the complement of adenosine receptors that are present. There is little evidence that adenosine is a neurotransmitter; rather, it appears to be a neuromodulator that is released in some unconventional ways to regulate and modulate neuronal activity. A current challenge in this field is to better define the mechanisms underlying the release of adenosine evoked by pathological and nonpathological stimuli. These kinds of studies should help to clarify the role of adenosine as a signaling agent in the brain and to relate this function to its other actions such as neuroprotection.

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